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Tumor Necrosis Factor Alpha mRNA and Protein Are Present in Human Placental and Uterine Cells at Early and Late Stages of Gestation

Hua-Lin Chen, Yaping Yang, Xiao-Ling Hu, Krishna K. Yelavarthi, James L. Fishback, and Joan S. Hunt

From the Department of Pathology, University of Kansas Medical Center, Kansas City, Kansas

Tumor necrosis factor alpha (TNF-α), a polypeptide that regulates cellular growth and modulates the synthesis of various cell surface and secreted molecules, has been identified in the pregnant uterus. To determine which specific cells transcribed and translated this gene, extraembryonic fetal tissues (placenta and membranes) and uterine tissue from early and late stages of gestation were analyzed for TNF-α mRNA by* in situ *hybridization using biotinylated antisense and sense TNF-α probes, and for immunoreactive TNF-α using two monoclonal antibodies. Tumor necrosis factor-alpha transcripts and protein were identified in both extraembryonic and maternal cells. In first-trimester placental villi, TNF-α mRNA was present in syncytiotrophoblast but was low to absent in cytotrophoblast and villous stromal cells. Decidual and epithelial cells in maternal tissues contained TNF-α transcripts. In term placentas, both syncytiotrophoblast and villous stromal cells contained TNF-α mRNA, and transcripts were present in maternal cells in the decidua adjacent to the extraplacental membranes. In both first-trimester and term tissues, coincident expression of TNF-α mRNA and immunoreactive TNF-α was demonstrated. The results of this study show that TNF-α is synthesized by cells in both extraembryonic membranes and maternal tissues during human gestation and that transcription in specific types of cells is influenced by gestational age. These observations are consistent with a major role for TNF-α in the dynamic developmental events of human pregnancy. (Am J Pathol 1991, 139:327–335)

Polypeptide factors that modulate cellular growth and differentiation are abundant in the pregnant uterus, and these factors are often produced locally by extraembryonic and uterine cells. Human placental trophoblast cells transcribe the interleukin-2[1] and colony-stimulating factor-GM genes,[2] and ovine trophoblast cells transcribe an alpha-interferonlike gene.[3] Mouse uterine decidual and epithelial cells contain transforming growth factor beta 1 mRNA (TGF-β 1),[4] and colony-stimulating factor-1 (CSF-1) transcripts are found in mouse uterine epithelial cells.[5–7] In many instances, immunologic or biologically active cytokines have been identified in these tissues.[4,5,8,9] In the case of CSF-1, coincidence of cytokine synthesis and the expression of cytokine receptors has been documented.[6,7]

Tumor necrosis factor alpha (TNF-α) is a 17-kd polypeptide that is active at picomolar concentrations and is associated with inflammatory processes. Originally identified as a product of activated macrophages that inhibited tumor cell proliferation,[10,11] TNF-α is now known to be produced by other types of cells[12,13] and to have pleiotrophic effects, modulating cellular growth, differentiation, and the synthesis of various substances.[14,15] Tumor necrosis factor-alpha might be one of the cytokines that influence human embryogenesis. Biologically active TNF-α has been identified in amniotic fluid and in supernatant culture media from human decidual and placental explants.[16,17] Radiolabel experiments have shown receptors for TNF-α in human term placental homogenates,[18] and TNF-α receptor mRNA has been reported in human term placenta.[19]

The role of TNF-α in human development is unknown, but identification of the specific cells that transcribe the TNF-α gene during the course of gestation might provide clues to its functions. Because many types of maternal and fetal cells are intermixed in these tissues, results ob-

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Address reprint requests to Joan S. Hunt, PhD, Department of Pathology, University of Kansas Medical Center, 39th St. and Rainbow Blvd., Kansas City, KS 66103.

tained by Northern and Western blotting could be subject to misinterpretation. We therefore analyzed semiserial sections of paraformaldehyde-fixed extraembryonic tissues and decidua taken from early and late stages of gestation directly by using *in situ* hybridization to identify TNF-α mRNA and immunohistology to localize the protein.

The results of this study provide the first evidence in humans that extraembryonic fetal cells and maternal cells transcribe the TNF-α gene and translate the mRNA into protein. The data presented suggest alterations in TNF-synthesizing cell populations as pregnancy progresses, and higher levels of this potent cytokine at term.

Materials and Methods

Tissues

Discarded tissues from elective first-trimester pregnancy terminations and term deliveries (caesarean sections) were acquired under a protocol approved by the institutional Human Subjects Committee. Placentas in first-trimester tissues (estimated date of gestation, 8 to 12 weeks; n = 4), term placentas (n = 4), and term extraplacental membranes (n = 4) were dissected into 1-cm^3 portions within 30 minutes after delivery. Tissue blocks of term placentas contained two to three samples taken from different cotyledons. Tissues were fixed overnight at 4°C in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2). Tissues were embedded in paraffin at low temperature, and 5-μ sections were placed onto poly-L-lysine–coated slides. Semiserial sections of these tissues were used for *in situ* hybridizations and immunocytochemical studies. Additional specimens of first-trimester placenta, term placenta, and term extraplacental membranes (n = 2 per type) were flash frozen in liquid N_2, sectioned by cryostat, and tested by immunohistology.

Probe Preparation and In Situ Hybridizations

The TNF-α probe, a 298-bp *Eco*R1-*Hind*III fragment of the murine TNF-α gene[20,21] subcloned into the transcription vector pGEM3 (Promega Biotec, Madison, WI) was a gift from Dr. C. Martens (DNAX, Palo Alto, CA). The high degree of homology between human and murine TNF-α genes allowed cross-hybridization.[14,15] The insert was excised using *Eco*R1 and *Hind* III restriction enzymes, and was verified for size by agarose gel electrophoresis. After linearization of the plasmid, biotinylated antisense and sense RNA probes were synthesized by *in vitro* transcription with Biotin 21-UTP (Clontech, Palo Alto, CA) using T7 and SP6 RNA polymerases (Promega) and ^{3}H-GTP as tracer for estimating probe concentrations. Purified biotinylated RNA probes were used in a hybridization mixture that contained 50% deionized formamide, 10% dextran sulfate, 2× SSC (20× SSC is 3M NaCl, 0.3M Na_3 citrate) 300 μg/ml yeast tRNA (Sigma Chemical Co., St. Louis, MO), and 30 units of RNase inhibitor (Promega). The final hybridization mixtures of sense and antisense versions of the probe contained equal cpm and equal amounts of RNA (0.2 to 0.6 μg/ml).

In situ hybridizations were performed using the method of Lawrence and Singer[22] as previously described[23] with some modifications. Deparaffinized tissue sections were prehybridized for 10 minutes at room temperature (RT) in PBS containing 5 mmol/l (millimolar) $MgCl_2$, then were incubated for 10 minutes at 65°C in 50% formamide, 2× SSC. The sections were overlaid with 50 μl of a hybridization cocktail and coverslipped, then were incubated overnight at 37°C in a humidified chamber. The tissue sections were washed for 30 minutes at 37°C in 50% formamide, 2× SSC, then in 50% formamide, 1× SSC, followed by several washes in 1× SSC. A final high-stringency wash was performed by incubating the slides for 30 minutes at 65°C in 0.1× SSC. The slides were washed for 10 minutes each in PBS containing 0.5% Triton X-100 and PBS at RT. Hybridization was detected with strepavidin-alkaline phosphatase and a nitroblue tetrazolium/bromo chloro indoyl phosphate substrate (Promega). The tissues were counterstained with methyl green. Some tissue sections were pretreated with 100 μg/ml of pancreatic RNase A (Sigma) for 30 minutes at 37°C. The RNase was inactivated with RNase inhibitor (2 μl/600 μl PBS), and the slides were washed extensively in PBS containing 5 mmol/l $MgCl_2$. In other control preparations, tRNA (Sigma) was substituted for the biotinylated probes.

Immunocytochemical Staining

Immunoreactive TNF-α in paraffin sections was identified with two mouse monoclonal antibodies (MAbs) to recombinant human TNF-α, one obtained from Olympus (clone F12, IgG_1) (Lake Success, NY) and one that was a gift from Genentech (TNF-E, Lot ≠5890-90, IgG_1) (South San Francisco, CA). Cytokeratin intermediate filaments were identified with PKK-1, LabSystems, Chicago, Ilinois, using the manufacturer's protocol for paraffin sections, and macrophages were identified with DAKO-MAC 387 (Dakopatts, Santa Barbara, CA). Deparaffinized, rehydrated tissue sections were first blocked with normal serum to prevent nonspecific binding, then the sections were in-

cubated with either specific antibody or normal mouse IgG at 10 μg/ml for 3 hours at 37°C in a humidified chamber. For inhibition experiments, 1 μg of anti–TNF-α was incubated for 1 hour at RT with 10 μg of recombinant human TNF-α (Genentech) in 100 μl of PBS. Binding was detected with an anti-mouse IgG kit from Zymed (San Francisco, CA). Endogenous peroxidase was quenched after the step that employed the biotinylated anti-mouse IgG reagent by incubating for 30 minutes in 0.3% H_2O_2 in methanol. The tissues were counterstained with Gill's hematoxylin. Cryostat sections of frozen tissues were dried for 1 hour, fixed for 7 minutes in cold acetone, rehydrated in PBS, and then were tested with TNF-E and normal mouse IgG as described above.

Results

TNF-α mRNA and Immunoreactive TNF-α in First-trimester Tissues

Samples taken from the first trimester of gestation contained a mixture of extraembryonic and maternal tissues and cells. Placental villi were composed of an outer layer of syncytiotrophoblast, an underlying layer of cytotrophoblast cells, and several types of mesenchymal cells (fibroblasts, macrophages, endothelial cells) in the central villous stroma. Maternal tissues contained large decidual cells, epithelia, endothelial cells, and leukocytes.

Figure 1a shows that TNF-α transcripts were present in first-trimester placental cells. RNA in intermittent stretches of syncytiotrophoblast hybridized to the antisense TNF-α probe but not to the sense version of the same probe (Figure 1b). Specific messages were low to undetectable in placental cytotrophoblast and were essentially absent in villous mesenchymal cells. Fragments of embryonic tissues (brain, intestine) present in these samples were negative (not shown). Syncytiotrophoblast stained intensely with anti–TNF-α but was negative with normal mouse IgG (Figure 1c, d). Immunoreactive TNF-α was present in cytotrophoblast cells, whereas villous mesenchymal cells were less strongly stained, as shown in Figure 1c and d.

Figures 2a and b show that large decidual cells in maternal tissues contained TNF-α transcripts and were positive with anti–TNF-α. Semiserial sections of tissues tested with anti-cytokeratin and an anti-macrophage reagent eliminated the possibility that these positive decidual cells were interstitial cytotrophoblastic cells or macrophages (Figure 2c, d). Epithelial cells lining the maternal blood spaces (endovascular trophoblast cells) also contained TNF-α mRNA and protein (Figure 2a, b), whereas endothelial cells and leukocytes were generally negative.

TNF-α mRNA and Immunoreactive TNF-α in Term Placentas

Term placentas differed from first-trimester tissues in that syncytial trophoblast predominated, villous cytotrophoblastic cells were uncommon and difficult to identify by morphology, and maternal tissue was represented only by a few leukocytes in the blood spaces.

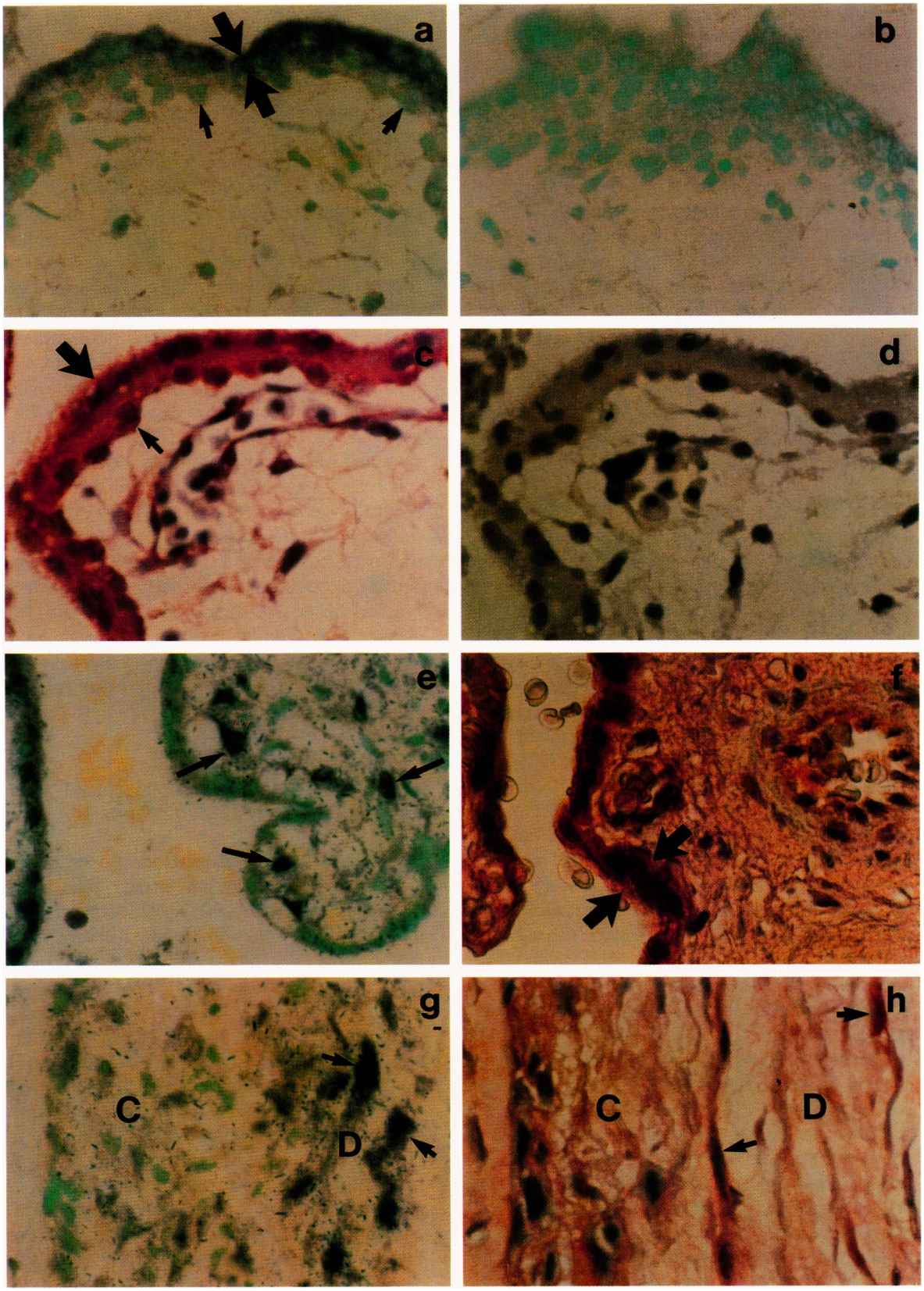
Tumor necrosis factor-alpha mRNA was present in syncytiotrophoblast in all four samples of term placenta. Although specimens from each placenta were similar, in two placentas, TNF-α transcripts were low in syncytiotrophoblast (Figure 1e), whereas in the other two placentas, syncytiotrophoblast contained high levels of TNF-α messages (Figure 3). In one of the latter samples, hybridization to syncytiotrophoblast was confined to small-diameter villi. Unlike first-trimester placental villi, mesenchymal cells in term placental villi were positive with the antisense TNF-α riboprobe; in all four samples, TNF-α transcripts were found in these cells (Figures 1e, 3a).

Identification of the cells that contained immunoreactive TNF-α was more difficult in term than in first-trimester tissues because the cytokine was present throughout the placental villous stroma, and was often found in maternal and fetal blood spaces (compare Figure 1c with 1f). Syncytiotrophoblast was usually positive with anti–TNF-α (Figure 1f). As with the *in situ* hybridizations for TNF mRNA, there was variability among the placentas that were tested for the protein, with some placentas staining more strongly than others.

TNF-α mRNA and Immunoreactive TNF-α in Term Extraplacental Membranes

At term, the extraplacental membranes are composed of four distinct tissues: 1) the amnion membrane, a single-cell layer of epithelium residing closest to the embryo, 2) multiple layers of fibrous tissue interspersed with a few mesenchymal cells, 3) the chorion membrane composed of cytotrophoblastic cells, and 4) maternal decidua.

Tumor necrosis factor-alpha mRNA was rarely present in amnion epithelial cells or fetal mesenchymal cells. Figure 1g shows that low levels of specific mRNA were identified in chorion membrane cytotrophoblast cells, and high levels of TNF-α transcripts were present in some cells in the adjacent maternal decidua. As in samples of term placenta, immunoreactive TNF-α was present in the stroma and maternal blood spaces in these membranes. Figure 1h shows that the cytokine was present in cytotrophoblastic cells in the chorion membrane, and that staining was somewhat more intense with cells in the adjacent decidua.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Controls

The use of sense probes in the same experiments in which antisense probes were tested were the primary controls for specific hybridization, and were negative. Internal controls were provided by positive and negative cells within each tissue section hybridized with the antisense probe. Preincubation of the tissue sections with RNase A and substitution of tRNA for the biotinylated probes was also done on all of the tissues used in this study, and all were negative.

In all of the fixed tissues that were evaluated, the two MAbs to TNF-α gave identical results, and normal mouse IgG controls were always negative. Two additional types of controls were performed. First we tested the specificity of the antibodies on paraffin sections after incubating with human recombinant TNF-α. Binding of both antibodies was completely abrogated by preincubation with TNF-α. Next immunohistology was performed on frozen tissues, where staining procedures are usually more sensitive. Unexpectedly these tests showed only low binding of anti–TNF-α to extraembryonic cells in first-trimester placentas and term membranes. Anti–TNF-α reacted strongly with maternal cells in placenta-associated fibrin and with some villous mesenchymal cells in term placental villi (not shown).

Table 1 gives a summary of the findings obtained by *in situ* hybridization and immunocytochemistry. All of the cells that contained TNF-α transcripts also contained immunoreactive TNF-α, but the protein was found in some additional cells as well as in acellular stroma. Although both fetal and maternal cells transcribed and translated the TNF gene, the predominant cell populations shifted during the course of pregnancy.

Discussion

This study provides the first evidence for local transcription and translation of the TNF-α gene by cells in human tissues during pregnancy. Both extraembryonic and maternal cells contained TNF-α transcripts and protein. These findings might have been predicted; placental and uterine cells produce many polypeptide growth factors,[1–9] and several studies suggest that TNF might be among these factors.[16–19,24,25]

Our results indicated temporal alterations in the cell populations that were TNF-α positive. Comparisons of staining intensities among various types of cells in the same tissues and among tissues from various stages of gestation allow only semiquantitative measures of differences. However, each *in situ* hybridization and immunocytochemical experiment, included samples of first-trimester tissues, term placenta, and term membranes. In placentas from early gestation, TNF-α mRNA and protein predominated in syncytiotrophoblast, whereas in mature placentas other types of cells participated in transcription and translation. Villous mesenchymal cells, many of which are macrophages,[26–30] appeared to gain a greater capacity to transcribe and translate the TNF-α gene as gestation progressed. Temporal alterations in cytokine-synthesizing cells during pregnancy have been noted in other studies. For example, TGF-β mRNA is prominent in murine luminal epithelium at implantation, and in decidual cells on subsequent days.[4]

Gestation-related fluctuations in cytokine synthesis are also well documented.[5–7] The results of this study suggested that more TNF-α may be present at late than at early stages of pregnancy, inasmuch as term tissues were often saturated with immunoreactive TNF-α. The TNF-α may have been synthesized by either extraembryonic or maternal cells, but it is worthy of note that staining intensities of maternal cells in both the *in situ* hybridizations and immunohistologic experiments were higher in term than in first-trimester tissues.

The ability of the two MAbs to recognize the cytokine more readily in fixed than in frozen tissues was unexpected. Specificity of the antibodies was clearly documented by inhibition experiments, so perhaps some conformational change occurred in the molecules during fixation that facilitated recognition by antibodies generated to recombinant TNF-α. Cells in frozen tissues that stained strongly with anti–TNF-α were located in areas known to be rich with macrophages.[28] These cells express class II

Figure 1. *TNF-α mRNA and immunoreactive TNF-α in human placental and uterine cells. Blue/purple staining identifies mRNA-positive cells (green counterstain). Red/brown staining identifies immunoreactive TNF-α (blue counterstain). Original magnification, ×312.* **a**: *First-trimester placenta, antisense probe. The antisense TNF-α probe hybridized to RNA in syncytiotrophoblast (large arrows) but not cytotrophoblast cells (small arrows) in cross-sections of placental villi.* **b**: *First-trimester placenta, sense probe. In a semiserial section of the same tissue, no hybridization was obtained.* **c**: *First-trimester placenta, anti–TNF-α (F12). Both syncytiotrophoblast (large arrows) and cytotrophoblast cells (small arrows) stained strongly with the antibody, but villous stromal cells were stained only lightly.* **d**: *First-trimester placenta normal mouse IgG. In a semiserial section of the same tissue, the control was negative.* **e**: *Term placenta, antisense probe. In cross sections of placental villi, RNA in mesenchymal cells hybridized with the antisense TNF-α probe. The sense probe did not hybridize to these or other cells in term placentas.* **f**: *Term placenta, anti–TNF-α (F12). Immunoreactive TNF-α was present in syncytiotrophoblast and throughut the villous stroma. In semiserial sections, normal mouse IgG did not bind to these or other placental cells.* **g**: *Term membranes, antisense probe. Cells in the maternal decidua (D) contained high levels of TNF-α mRNA, and cytotrophoblast cells in the chorion membrane (C) were weakly positive. The sense probe did not hybridize to any cells in a semiserial section of the same tissue.* **h**: *Term membranes, anti–TNF-α (F12). Immunoreactive TNF-α was present throughout the stroma of the membranes. The antibody bound to cytotrophoblast cells in the chorion membrane (C), and, somewhat more strongly, to cells in the maternal decidua (D). No binding of normal mouse IgG was observed in a semiserial section of the same tissue.*

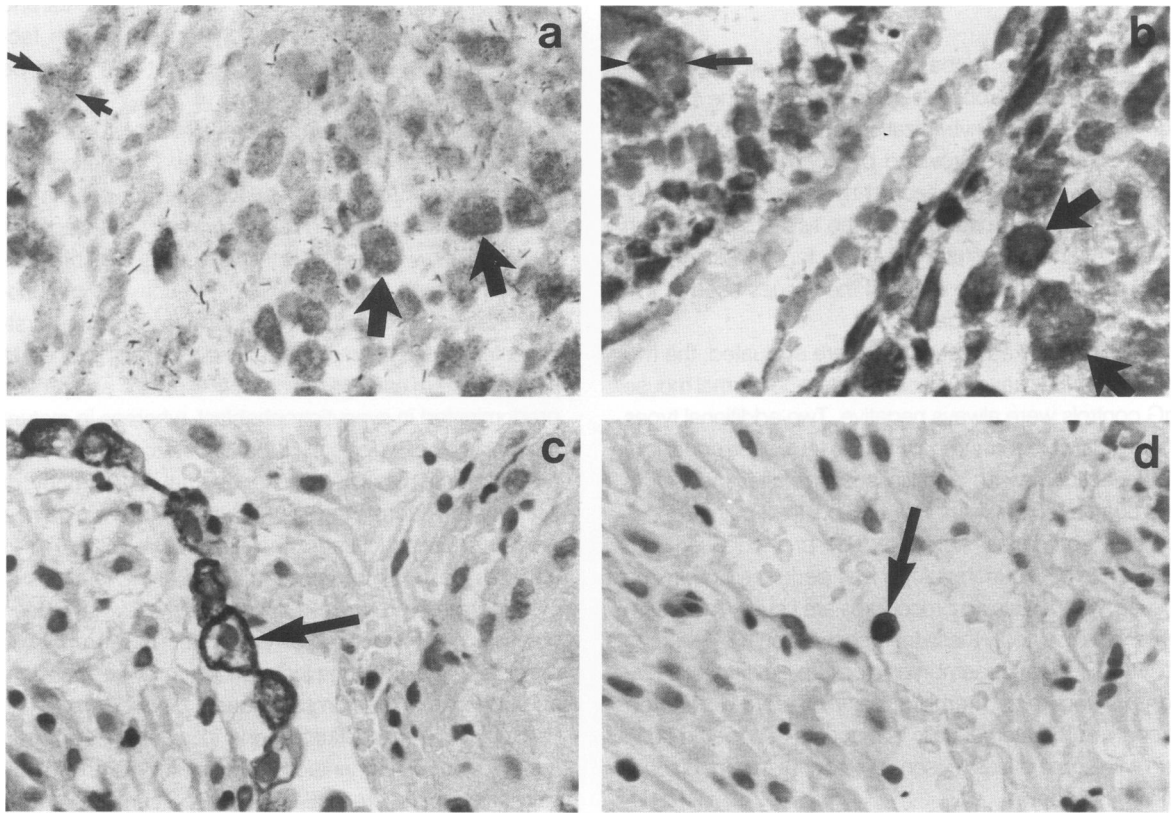

Figure 2. *TNF-α mRNA and protein in first-trimester decidual cells.* **a:** *The antisense TNF-α probe hybridized to RNA in large decidual cells (large arrows) and epithelial cells (small arrows).* **b:** *Large decidual cells (large arrows) and epithelial cells (small arrows) were positive with the monoclonal antibody to TNF, F12.* **c:** *Staining with anti-cytokeratin failed to identify the large TNF-α mRNA-positive cells as interstitial cytotrophoblastic cells. The arrow marks cytokeratin-positive trophoblast.* **d:** *Staining with DAKO-MAC 387 failed to identify the TNF-α mRNA-positive cells in the decidua as macrophages. The arrow marks a positive blood monocyte. Original magnification, ×312.*

major histocompatibility antigens[27,28] and are therefore in the activated state associated with high synthesis of TNF-α.[10] Thus concentration differences may have accounted for our observation that these but not other cells were strongly stained. This study did not indicate that either the TNF-α probe or the antibodies recognized polymorphic variants of TNF.[31,32]

The functions of uteroplacental TNF-α remain a matter of speculation, but might include regulation of proliferation by cells in the implanted blastocyst. DNA synthesis by rapidly dividing human trophoblast-derived choriocarcinoma cells[33] and rat trophoblast cells taken from midgestation placentas[34,35] is diminished by TNF-α, whereas the cytokine has no effect on quiescent trophoblast cells

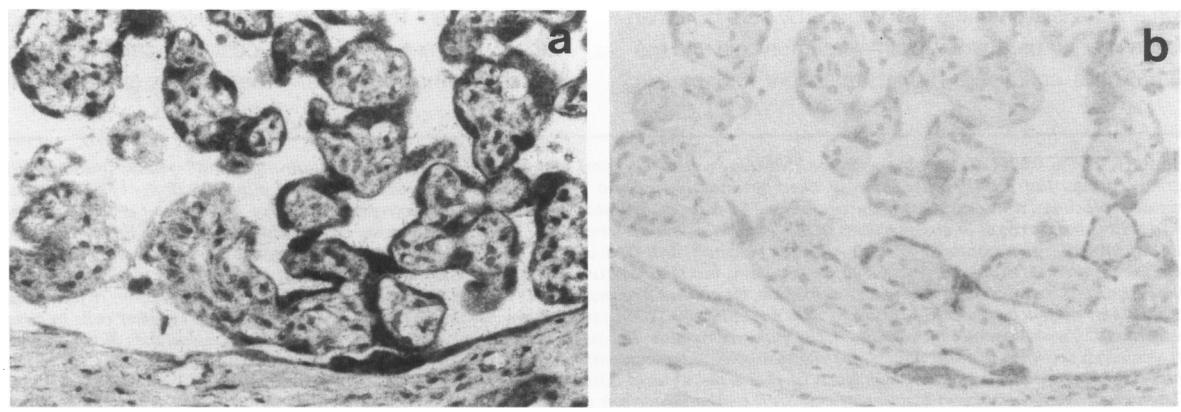

Figure 3. *TNF-α mRNA in term placental villi.* **a:** *The antisense TNF-α probe hybridized to RNA in syncytiotrophoblast and villous mesenchymal cells.* **b:** *In a semiserial section of the same tissue, no hybridization was obtained with the sense TNF-α probe. Original magnification, ×125.*

Table 1. *Summary of the* In Situ *Hybridization and Immunocytochemical Experiments on First-trimester Placentas, Term Placentas, and Term Extraplacental Membranes*

	TNF-a-mRNA	Immuno-reactive TNF-a
First-trimester tissues		
Placenta		
Syncytiotrophoblast	+*	+
Cytotrophoblastic cells	0	+
Villous mesenchymal cells	0	+/0
Acellular stroma	0	0
Decidua		
Decidual cells	+	+
Endovascular trophoblast	+	+
Endothelial cells	0	0
Term placentas		
Syncytiotrophoblast	+/0	+
Villous mesenchymal cells	+	+
Acellular stroma	0	+
Term extraplacental Membranes		
Amnion	0	0
Fetal mesenchymal cells	0	0
Acellular stroma	0	+
Chorion	+/0	+
Cells *in decidua*	+	+

* +, The majority of cells were positive; 0, the majority of cells were negative; +/0 indicates weak or inconsistent staining of cells.

from term murine placentas,[36] and is a growth stimulator for rat embryonic fibroblasts.[35] Possibly TNF-α has pleotrophic effects on blastocyst-derived cells, protecting the mother from excessive uterine invasion by rapidly proliferating trophoblast cells[37] while stimulating the growth of embryonic mesenchymal cells.

Effects on differentiation and the expression of various cell surface and secreted molecules also might be a function of this potent cytokine. The expression of class 1 major histocompatibility (MHC) antigens is required for recognition of infected cells by cytotoxic T lymphocytes, and for binding of various hormones and growth factors.[38–40] Tumor necrosis factor-alpha does not increase class I MHC expression by either murine[41] or rat trophoblast cells,[35] but enhances expression by rat embryonic fibroblasts.[35] Tumor necrosis factor-alpha also might influence tissue remodeling in the uterus by regulating collagenase synthesis,[42] promote angiogenesis,[43] protect against natural killer cells,[44] or limit lymphocyte infiltration into the uterus.[45] Our observation that term but not first-trimester placental stroma and blood spaces are suffused with TNF-α supports the hypothesis that this molecule plays a role in parturition.[17,46]

Studies of this type that document the transcription and translation of various cytokines in specific cells during the course of gestation are required for understanding the events of both normal and abnormal pregnancy. Infertility is a major health problem that could be related to inappropriate cytokine synthesis, as suggested by studies in mice,[47] and premature labor is known to be associated with higher than normal local synthesis of various cytokines.[46,48] These conditions might be amenable to treatment if normal patterns of synthesis and cellular requirements could be determined. This study establishes that one of the cytokines that must be considered is TNF-α.

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References

1. Boehm KD, Kelley MF, Ilan J, Ilan J: The interleukin 2 gene is expressed in the syncytiotrophoblast of the human placenta. Proc Natl Acad Sci USA 1989, 86:656–660
2. Uzumaki H, Okabe T, Sasaki N, Hagiwara K, Takaku F, Tobita M, Yasukawa K, Ito S, Umezawa Y: Identification and characterization of receptors for granulocyte colony-stimulating factor on human placenta and trophoblastic cells. Proc Natl Acad Sci USA 1989, 86:9323–9326
3. Farin CE, Imakawa K, Roberts RM: *In situ* localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. Mol Endocrinol 1989, 3:1099–1107
4. Tamada H, McMaster MT, Flanders KC, Andrews GK, Dey SK: Cell type-specific expression of TGF-β 1 in the mouse uterus during the periimplantation period. Mol Endocrinol 1990, 4:965–972
5. Pollard JW, Bartocci A, Arceci R, Orlofsky A, Ladner MB, Stanley ER: Apparent role of the macrophage growth factor, CSF-1, in placental development. Nature 1987, 330:484–486
6. Regenstreif LJ, Rossant J: Expression of the c-*fms* proto-oncogene and of the cytokine, CSF-1 during mouse embryogenesis. Dev Biol 1989, 133:284–294
7. Arceci RJ, Shanahan F, Stanley ER, Pollard JW: Temporal expression and location of colony-stimulating factor 1 (CSF-1) and its receptor in the female reproductive tract are consistent with CSF-1-regulated placental development. Proc Natl Acad Sci USA 1989, 86:8818–8822
8. Soubiran P1, Zapitelli J-P, Schaffar L: IL-2-like material present in human placenta and amnion. J Reprod Immunol 1987, 12:225–234
9. Flynn A, Finke JH, Hilfiker ML: Placental mononuclear phagocytes as a source of interleukin-1. Science 1982, 218:475–477
10. Beutler B, Mahoney J, Trang NL, Pekala P, Cerami A: Purification of cachectin, a lipoprotein lipase-suppressing hor-

mone secreted by endotoxin-induced RAW 264.7 cells. J Exp Med 1985, 161:984–995
11. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA, Shepard HM: Recombinant human tumor necrosis factor-α: Effects on proliferation of normal and transformed cells in vitro. Science 1985, 230:943–945
12. Robbins DS, Shirazi Y, Drysdale BE, Leiberman A, Shin HS, Shin ML: Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. J Immunol 1987, 139:2593–2597
13. Sawada M, Kondo N, Suzumura A, Marunouchi T: Production of tumor necrosis factoralpha by microglia and astrocytes in culture. Brain Res 1989, 491:394–397
14. Le J, Vilcek J: Biology of disease. Tumor necrosis factor and interleukin 1: Cytokines with multiple overlapping biological activities. Lab Invest 1987, 56:234–248
15. Beutler B, Cerami A: The biology of cachectin/TNF—a primary mediator of the host response. Annu Rev Immunol 1989, 7:625–655
16. Jaattela M, Kuusela P, Saksela E: Demonstration of tumor necrosis factor in human amniotic fluids and supernatants of placental and decidual tissues. Lab Invest 1988, 58:48–52
17. Casey ML, Cox SM, Beutler B, Milewich L, MacDonald PC: Cachectin/tumor necrosis factor-α formation in human decidua. J Clin Invest 1989, 83:430–436
18. Eades DK, Cornelium P, Pekala PH: Characterization of the tumor necrosis factor receptor in human placenta. Placenta 1988, 9:247–251
19. Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jerzy R, Dower SK, Cosman D, Goodwin RG: A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 1990, 248:1019–1023
20. Fransen L, Muller R, Marmenout A, Tavernier J, Van der Heyden J, Kawashima E, Chollet A, Tizard R, Van Heuverswyn H, Van Vliet A, Ruysschaert MR, Fiers W: Molecular cloning of mouse tumor necrosis factor cDNA and its eukaryotic expression. Nucleic Acids Res 1985, 13:4417–4429
21. Murray L, Martens C: The abnormal T lymphocytes in *lpr* mice transcribe interferongamma and tumor necrosis factor-α genes spontaneously *in vivo*. Eur J Immunol 1989, 19:563–565
22. Lawrence JB, Singer RH: Quantitative analysis of *in situ* hybridization methods for the detection of actin gene expression. Nucleic Acids Res 1985, 13:1777–1799
23. Yelavarthi KK, Fishback JL, Hunt JS: Analysis of HLA-G mRNA in human placental and extraplacental membrane cells by in situ hybridization. J Immunol 1991, 146:3840–3848
24. Crainie M, Guilbert L, Wegmann TG: Expression of novel cytokine transcripts in the murine placenta. Biol Reprod 1990, 43:999–1005
25. Witkin SS, Liu H-C, Davis OK, Rosenwaks Z: Tumor necrosis factor is present in maternal sera and embryo culture fluids during in vitro fertilization. J Reprod Immunol 1991, 19:85–93
26. Moskalewski S, Ptak W, Czarnik Z: Demonstration of cells with IgG receptor in human placenta. Biol Neonate 1975, 26:268–272
27. Hunt JS, King CR, Wood GW: Evaluation of human chorionic trophoblasts and placental macrophages as stimulators of maternal lymphocyte proliferation in vitro. J Reprod Immunol 1984, 6:377–391
28. Lessin DL, Hunt JS, King CR, Wood GW: Antigen expression by cells near the maternal-fetal interface. Am J Reprod Immunol Microbiol 1988, 16:1–7
29. Mues B, Langer D, Zwadlo G, Sorg C: Phenotypic characterization of macrophages in human term placenta. Immunology 1989, 67:303–307
30. Hunt JS: Macrophages in human uteroplacental tissues. A review. Am J Reprod Immunol 1989, 21:119–122
31. Jongeneel CV, Acha-Orbea H, Blankenstein T: A polymorphic microsatellite in the tumor necrosis factor alpha promoter identifies an allele unique to the NZW mouse strain. J Exp Med 1990, 171:2141–2146
32. Webb GC, Chaplin DD: Genetic variability at the human tumor necrosis factor loci. J Immunol 1990, 145:1278–1285
33. Berkowitz RS, Hill JA, Kurtz CB, Anderson DJ: Effects of products of activated leukocytes (lymphokines and monokines) on the growth of malignant trophoblast cells in vitro. Am J Obstet Gynecol 1988, 158:199–203
34. Hunt JS, Soares MJ, Lei M-G, Smith RN, Wheaton D, Atherton RA, Morrison DC: Products of lipopolysaccharide-activated macrophages (tumor necrosis factor-α, transforming growth factor-β) but not lipopolysaccharide modify DNA synthesis by rat trophoblast cells exhibiting the 80 kDa lipopolysaccharide-binding protein. J Immunol 1989, 143:1606–1613
35. Hunt JS, Atherton RA, Pace JL: Differential responses of rat trophoblast cells and embryonic fibroblast cells to cytokines that regulate proliferation and class I MHC antigen expression. J Immunol 1990, 145:184–189
36. Drake BL, Head JR: Murine trophoblast cells are not killed by tumor necrosis factor-α. J Reprod Immunol 1990, 17:93–99
37. Hunt JS: Cytokine networks in the uteroplacental unit: Macrophages as pivotal regulatory cells. J Reprod Immunol 1989, 16:1–17
38. Fehlmann M, Peyron J-F, Samson M, Van Obberghen E, Brandenburg D, Brossette N: Molecular association between major histocompatibility complex class I antigens and insulin receptors in mouse liver membranes. Proc Natl Acad Sci USA 1985, 82:8634–8657
39. Schreiber AB, Schlessinger J, Edidin M: Interaction between major histocompatibility complex antigens and epidermal growth factor receptors on human cells. J Cell Biol 1984, 98:725–731
40. Hunt JS, Andrews GK, Fishback JL, Feess M, Wood GW: Amnion membrane epithelial cells express class I HLA and contain class I HLA mRNA. J Immunol 1988, 140:2790–2795
41. King NJ, Maxwell LE, Kesson AM: Induction of class I major histocompatibility complex antigen expression by West Nile virus on gamma interferon-refractory early murine trophoblast cells. Proc Natl Acad Sci USA 1989, 86:911–915
42. Dayer JM, Beutler B, Cerami A: Cachectin/tumor necrosis factor (TNF) stimulates collagenase and PGE_2 production

by human synovial cells and dermal fibroblasts. J Exp Med 1985, 162:2163–2168

43. Frater-Schroder M, Risau W, Hallmann R, Gautschi P, Bohlen P: Tumor necrosis factor type a, a potent inhibitor of endothelial cell growth *in vitro,* is angiogenic *in vivo.* Proc Natl Acad Sci 1987, 84:5277–5281
44. Reiter Z, Rubinstein M: Interleukin-1 and tumor necrosis factor-α protect cells against natural killer cell-mediated cytotoxicity and natural killer cytotoxic factor. Cell Immunol 1990, 125:326–336
45. Jacob CO, Aiso S, Michie SA, McDevitt HO, Acha-Orbea H: Prevention of diabetes in nonobese diabetic mice by tumor necrosis factor (TNF): Similarities between TNF-α and interleukin 1. Proc Natl Acad Sci USA 1990, 84:968–972
46. Romero R, Manogue KR, Mitchell MD, Wu YK, Oyarzun E, Hobbins JC, Cerami A: Infection and labor IV. Cathectin-tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor. Am J Obstet Gynecol 1989, 161:336–341
47. Wegmann TG: Placental immunotrophism: Maternal T cells enhance placental growth and function. Am J Reprod Immunol Microbiol 1987, 15:67–70
48. Romero R, Avila C, Santhanam U, Sehgal PB: Amniotic fluid and interleukin-6 in preterm labor: Association with infection. J Clin Invest 1990, 85:1392–1400